

Figure 1A. Construction of JB-3120-2, a plasmid containing T₇ promoter H91A *hin47* (+ leader)

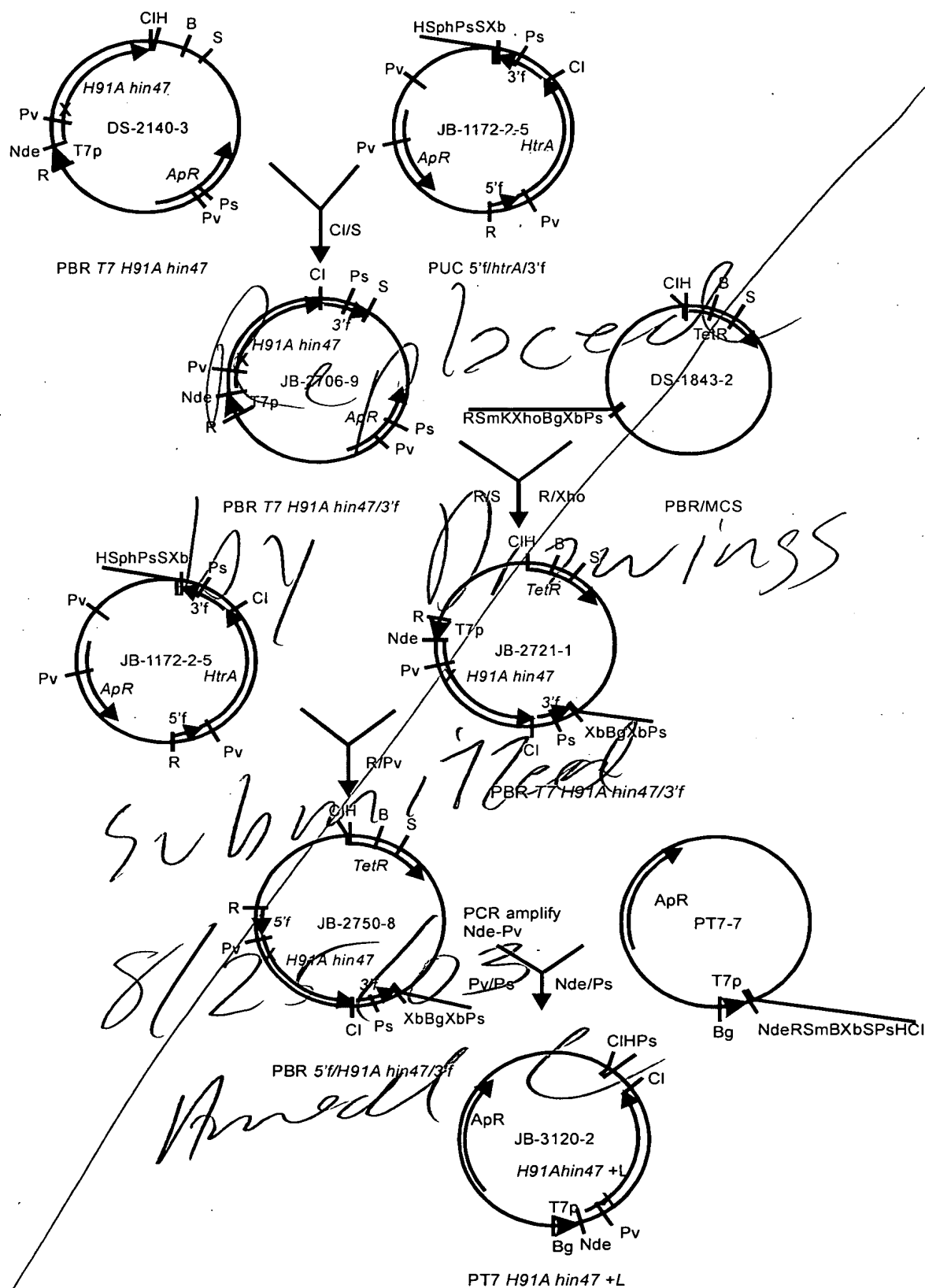
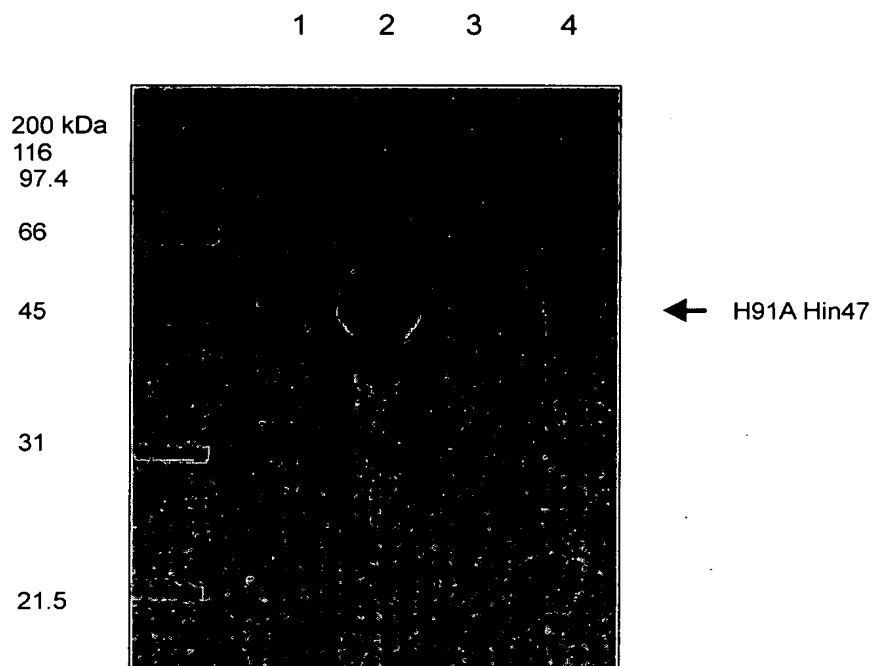


Figure 1B. Oligonucleotide primers to PCR amplify the Nde I-Pvu I fragment encoding the leader sequence of HtrA.

		Nde I												
			M	K	K	T	R	F	V	L	N	S		
5'	GGCCG	CATATG	AAAAAA	ACACG	TTTT	GTACT	AAATAGT	3'	6931.SL	SEQ ID No: 2				
										SEQ ID No: 1				
		Pvu I												
			F	K	F	F	F	G	D	R	F	A	E	Q
	TTTAAATTCTTCTTTGG	CGATCG	TTTT	GCCGAACAA										
3'	AAATTTAAGAAGAAACCGCTAGCAAACGGCTTGTT	5'	6932.SL	SEQ ID No: 5										
				SEQ ID No: 4										
				SEQ ID No: 3										

Figure 2. Production of H91A Hin47 +/- leader



1. H91A Hin47 (- leader) t_0
2. H91A Hin47 (- leader) t_4
3. H91A Hin47 (+ leader) t_0
4. H91A Hin47 (+ leader) t_4

Figure 3. Purification of H91A Hin47 (+ leader) from *E. coli*

***E. coli* Whole Cell**

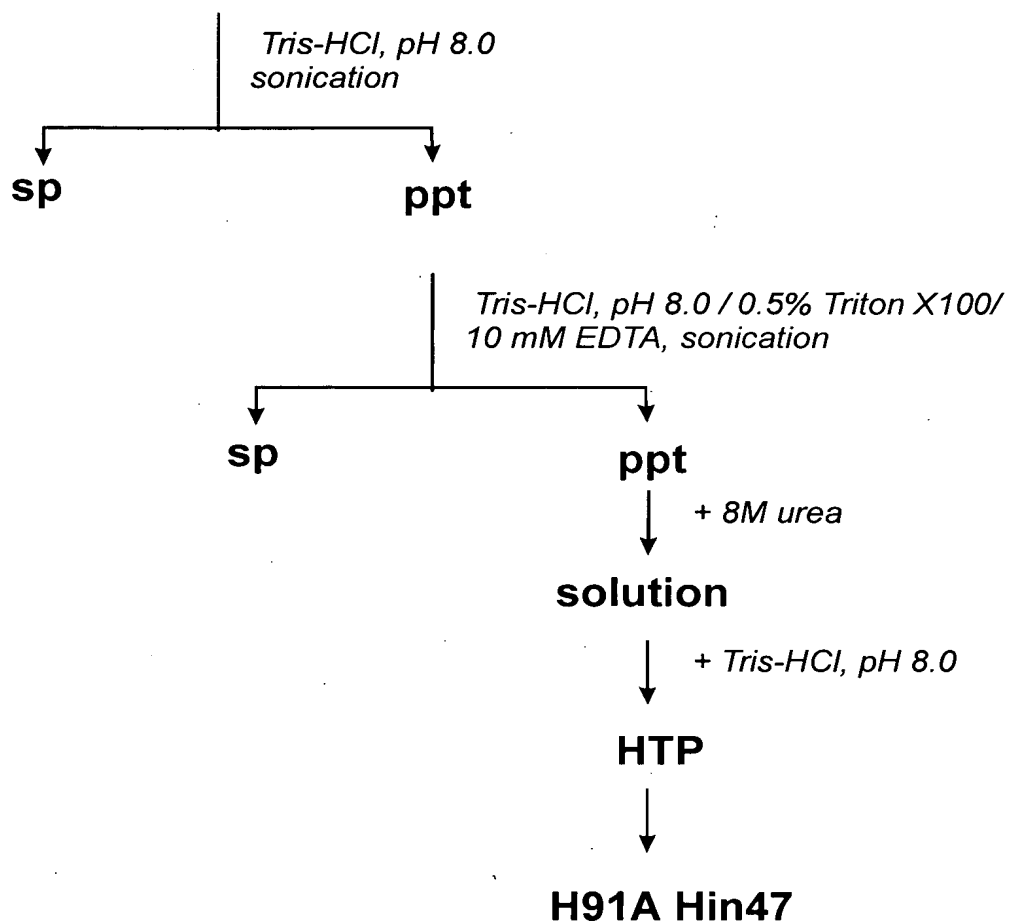
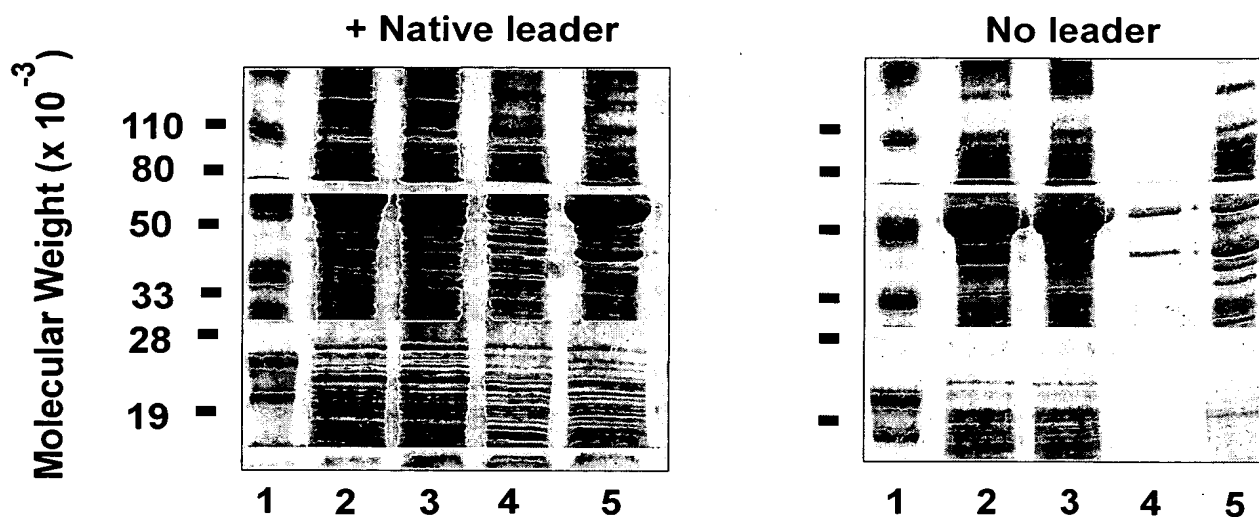
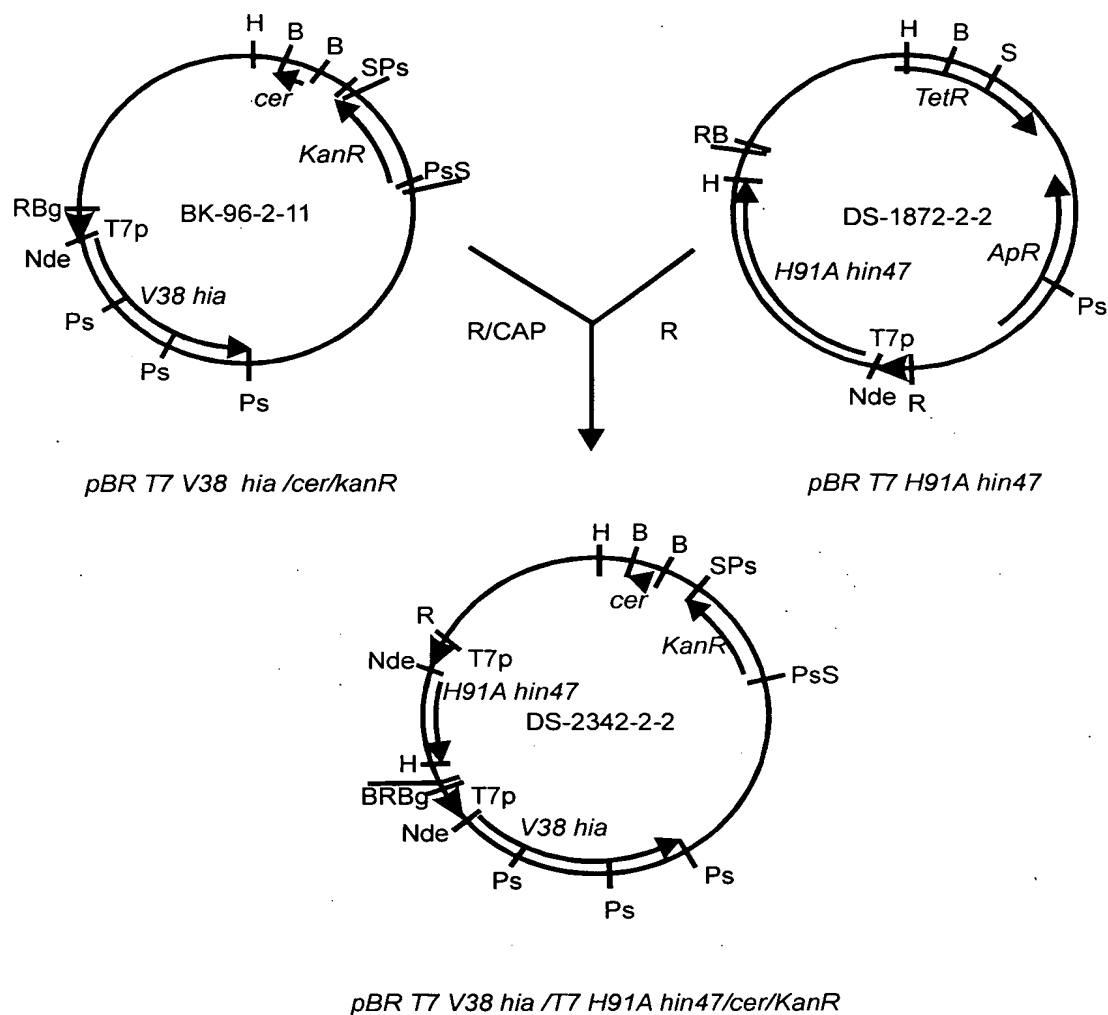


Figure 4. Extraction of H91A Hin47 +/- leader, produced in *E. coli*



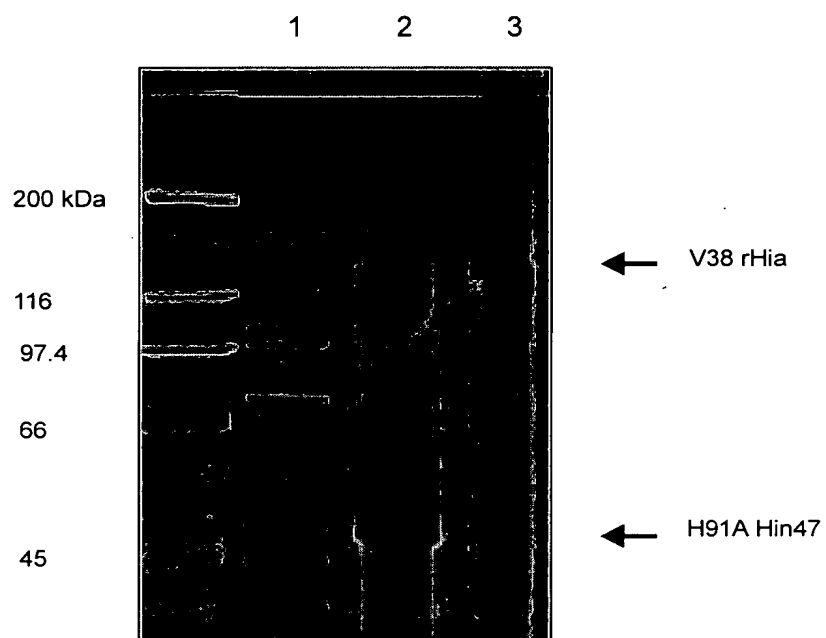
1. Pre-stained markers
2. *E. coli* whole cells
3. Soluble proteins in 50 mM Tris-HCl extraction
4. Soluble proteins in Tris/EDTA/Triton-100 extraction
5. Remaining pellets

Figure 5. Construction of DS-2342-2-2, a plasmid containing T7 H91A *hin47* and T7 V38 *hia*



Co-exprn fig 5
pDS-2342

Figure 6. Production of V38 rHia and H91A Hin47 when co-expressed



1. V38 rHia and H91A Hin47 t_0
2. V38 rHia and H91A Hin47 t_4
3. V38 rHia alone t_4

Co-exprn fig 6
V38hin

Figure 7. Purification of H91A Hin47 and V38 rHia from *E. coli*

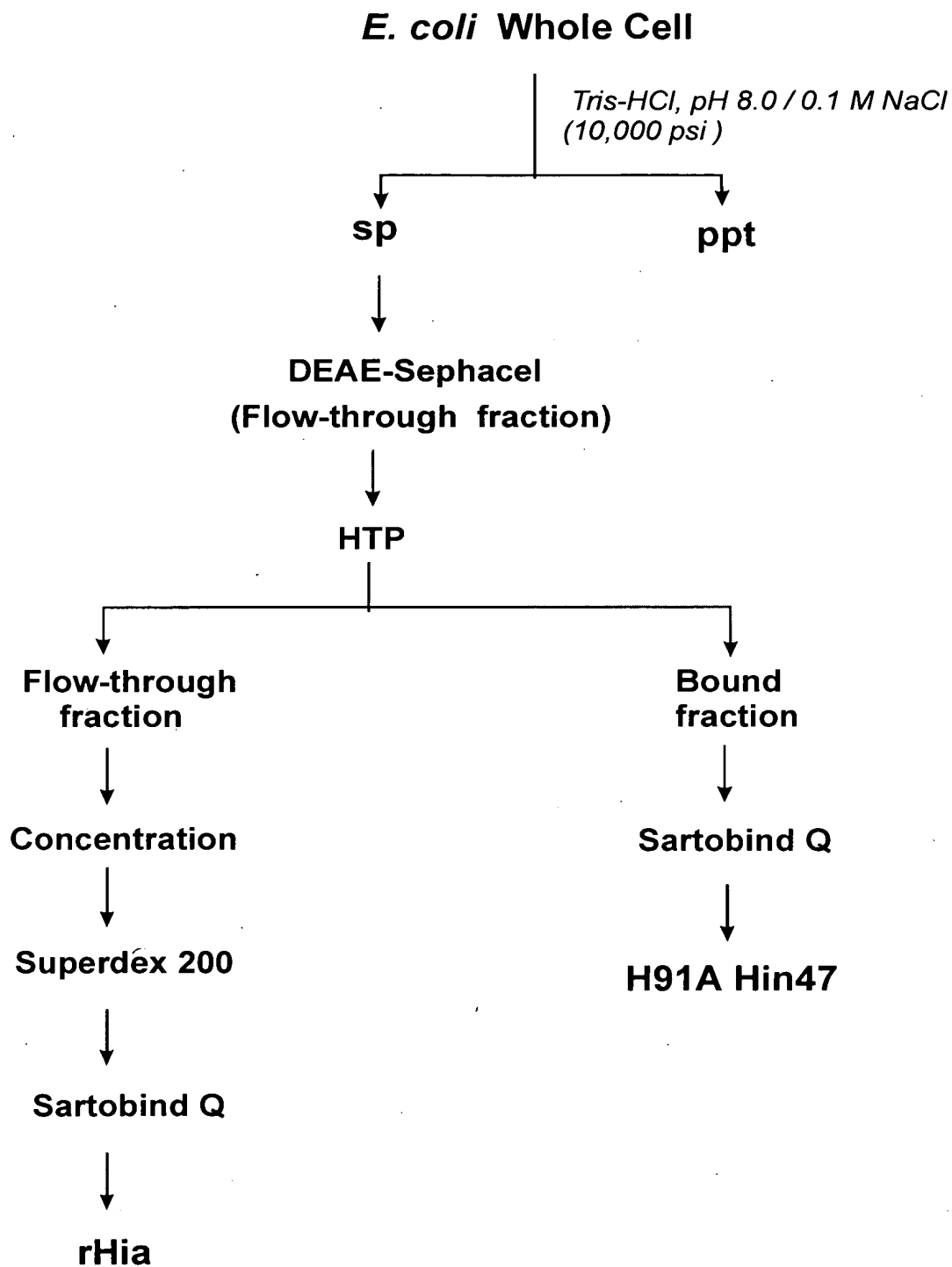
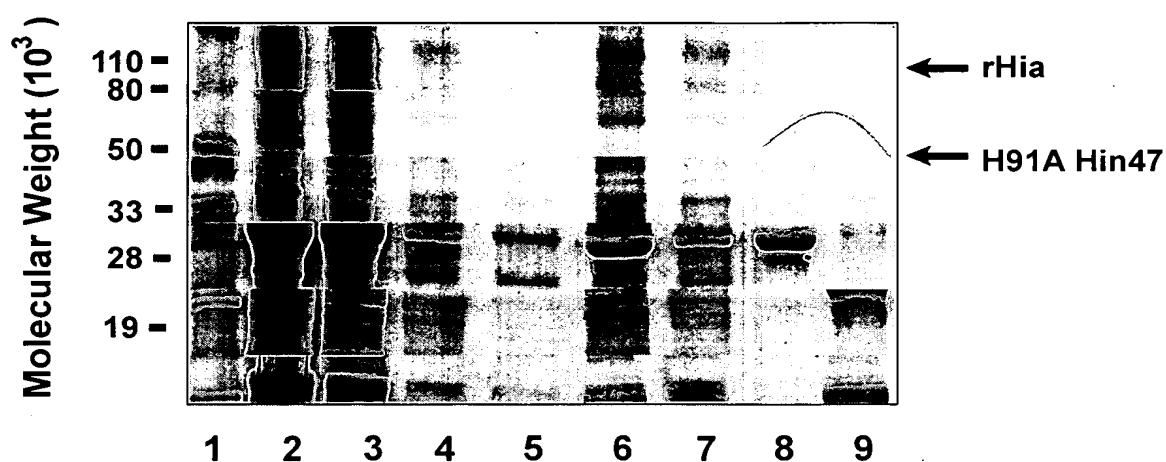
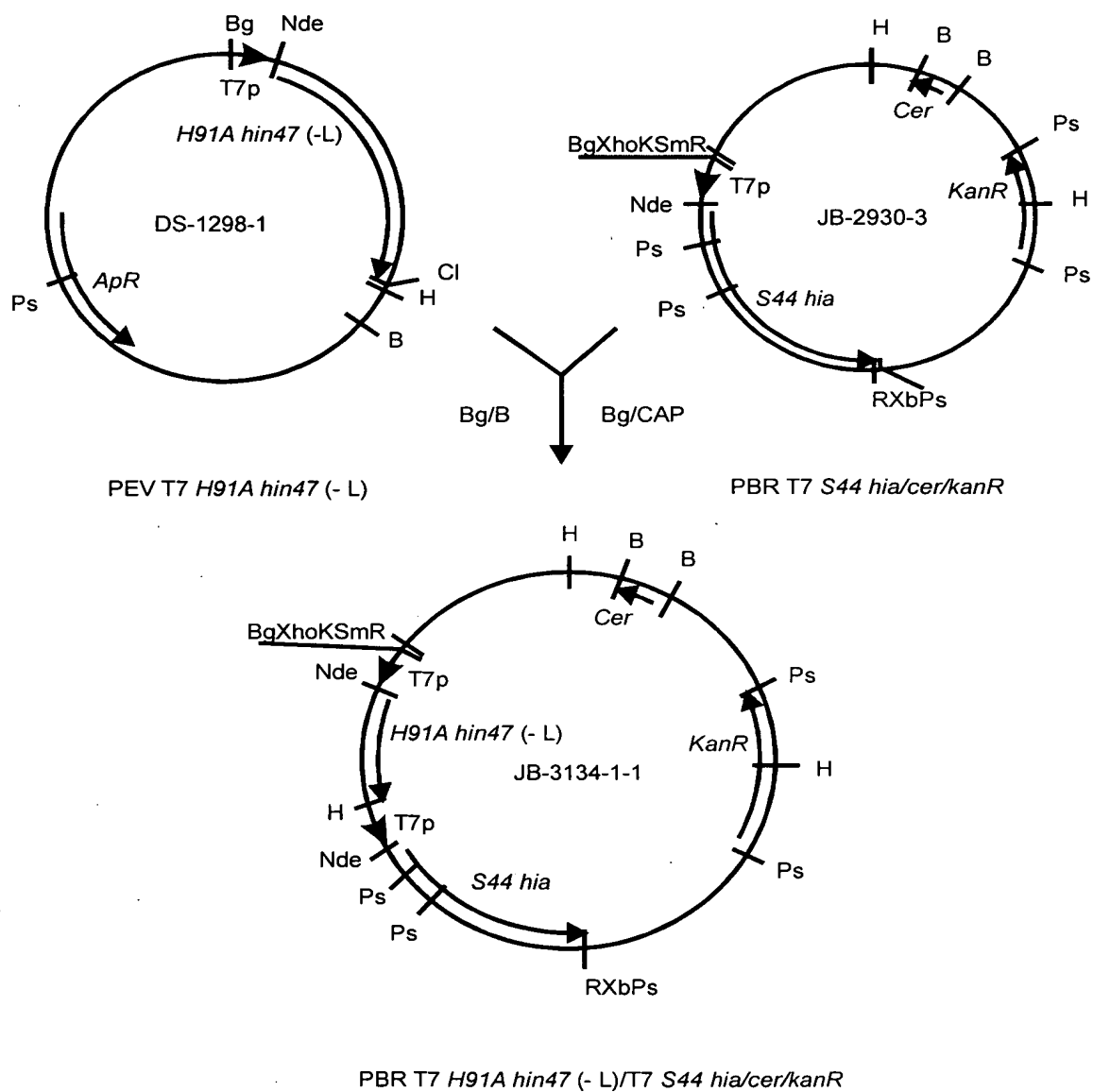


Figure 8. Purification of H91A Hin47 and V38 rHia from *E. Coli*



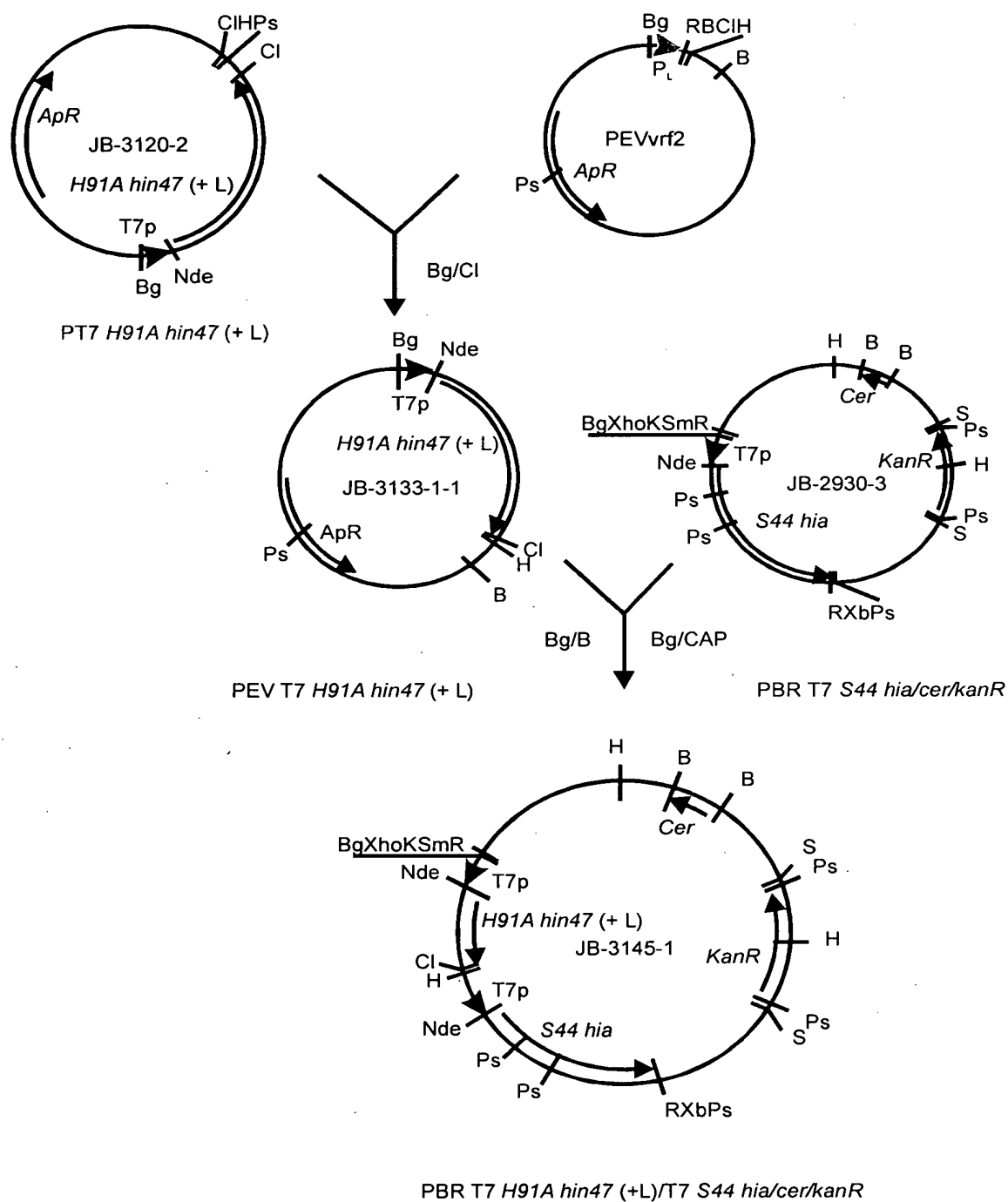
- | | |
|--|------------------------|
| 1. Prestained molecular weight markers | 6. DEAE flow-through |
| 2. <i>E. coli</i> whole cell lysate | 7. HTP flow-through |
| 3. Tris/NaCl extract | 8. Purified H91A Hin47 |
| 4. Tris/ Triton X-100 / EDTA extract | 9. Purified rHia |
| 5. Tris/ OG extract | |

Figure 9. Construction of JB-3134-1-1, a plasmid containing T7 *H91A hin47* (- leader) and T7 *S44 hia*



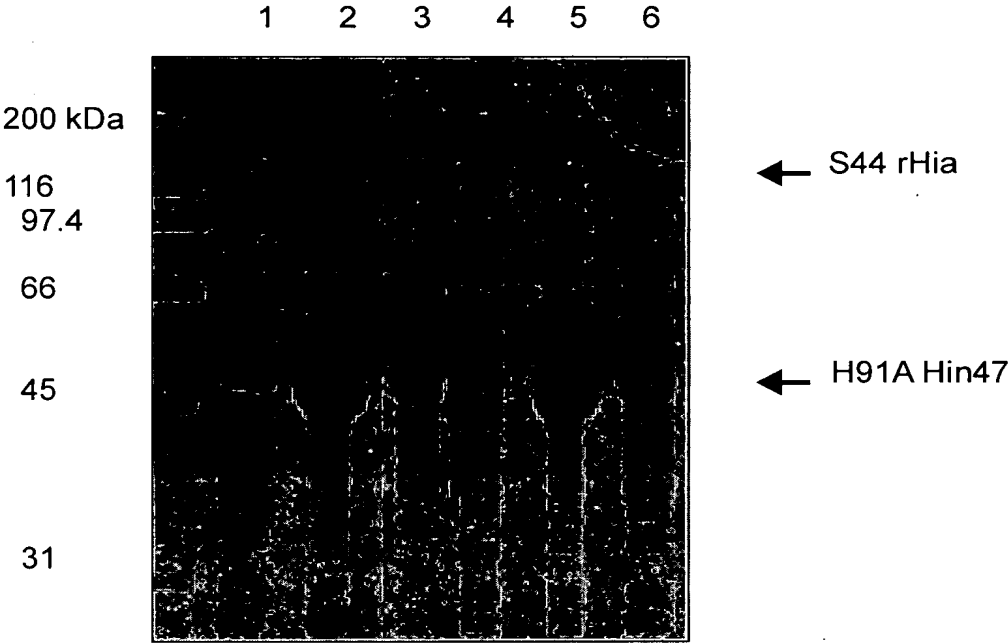
Co-expn fig 9
pJB-3134

Figure 10. Construction of JB-3145-1, a plasmid containing T7 *H91A hin47* (+ leader) and T7 *S44 hi*.



Co-exprn fig 10
pJB-3145

Figure 11. Production of S44 rHia and H91A Hin47 (+/-L), when co-expressed.



- | | |
|-------------------------------|-------|
| 1. H91A Hin47 (-L) | t_0 |
| 2. H91A Hin47 (-L) | t_4 |
| 3. H91A Hin47 (+L) | t_4 |
| 4. S44 rHia | t_4 |
| 5. H91A Hin47 (-L) + S44 rHia | t_4 |
| 6. H91A Hin47 (+L) + S44 rHia | t_4 |

Figure 12A. Construction of JB-3073R-1, a plasmid containing T7 *psaA* (leader) and T7 *H91A hin47*

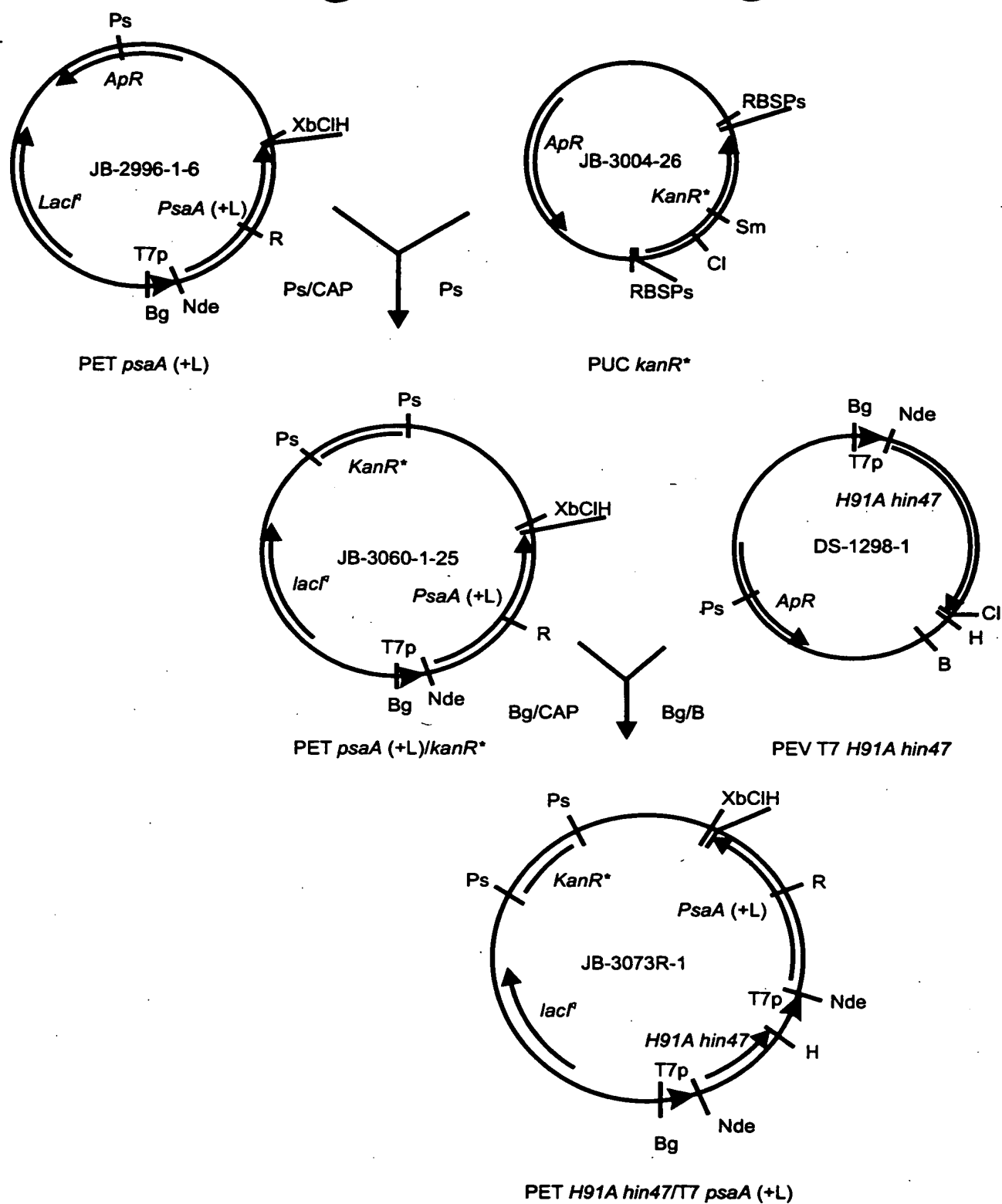


Figure 12B. Oligonucleotide primers to PCR amplify psaA (+ native leader)

Sense:

Nde I
 M K K L G T L L V L
 5' CGGGATCC CATATG AAAAAATTAGGTACATTACTCGTTCTC 3' 6850.SL
 Srd 10 no: 27
 Srd 10 no: 26

Antisense:

CATGTGAGCTGGCGTTTTTTAAGCTTGGCC
 3' GTACACTCGACCGCAAAAAA TTCGAA CCGG 5' 6852.SL
 Hind III
 Srd 10 no: 29
 Srd 10 no: 28

Figure 13A. Construction of JB-3090-1 and JB-3090-7 plasmids containing T7 *psaA* (- leader) and T7 *H91A hin47*

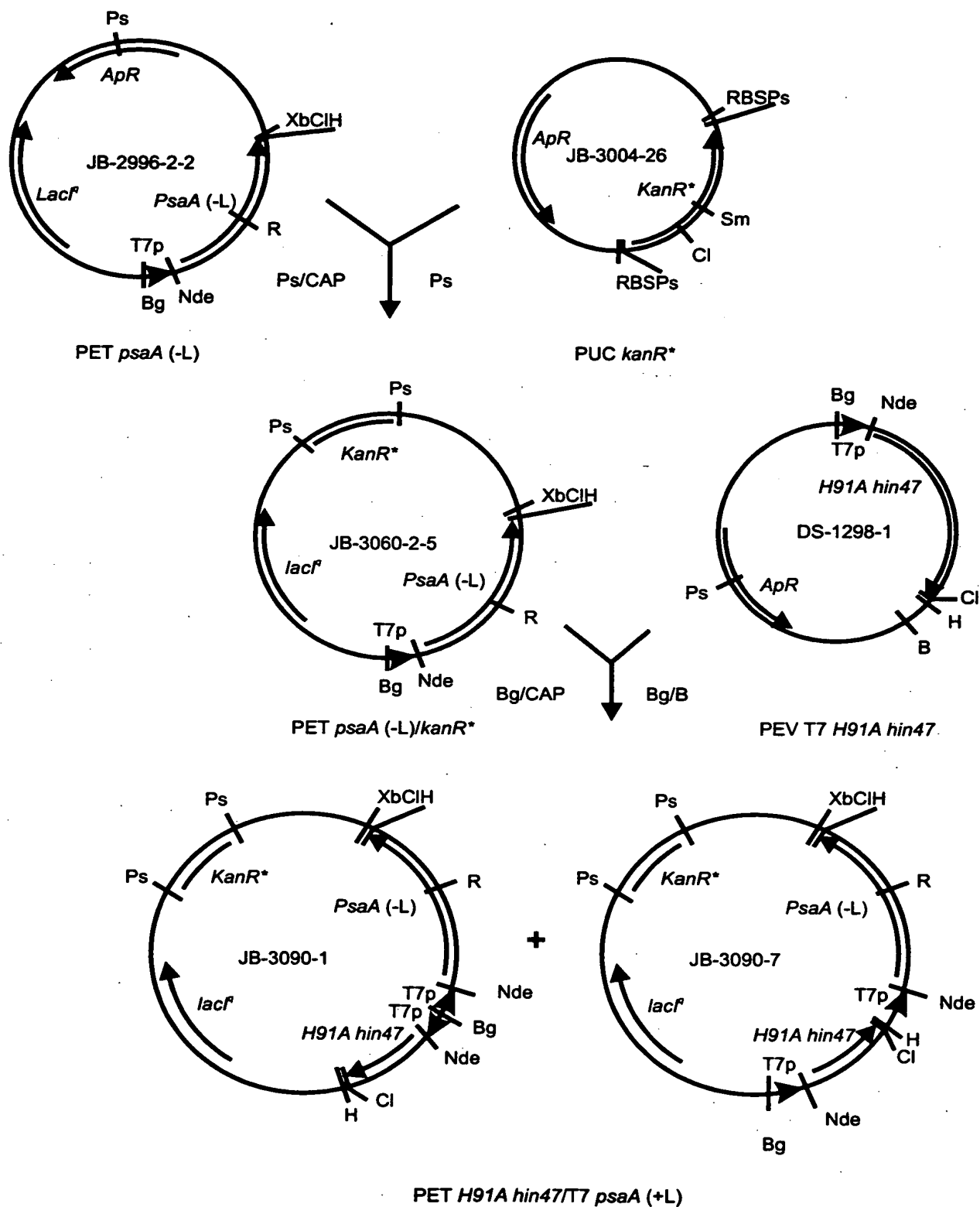


Fig 13B. Oligonucleotide primers to PCR amplify psaA (- leader)

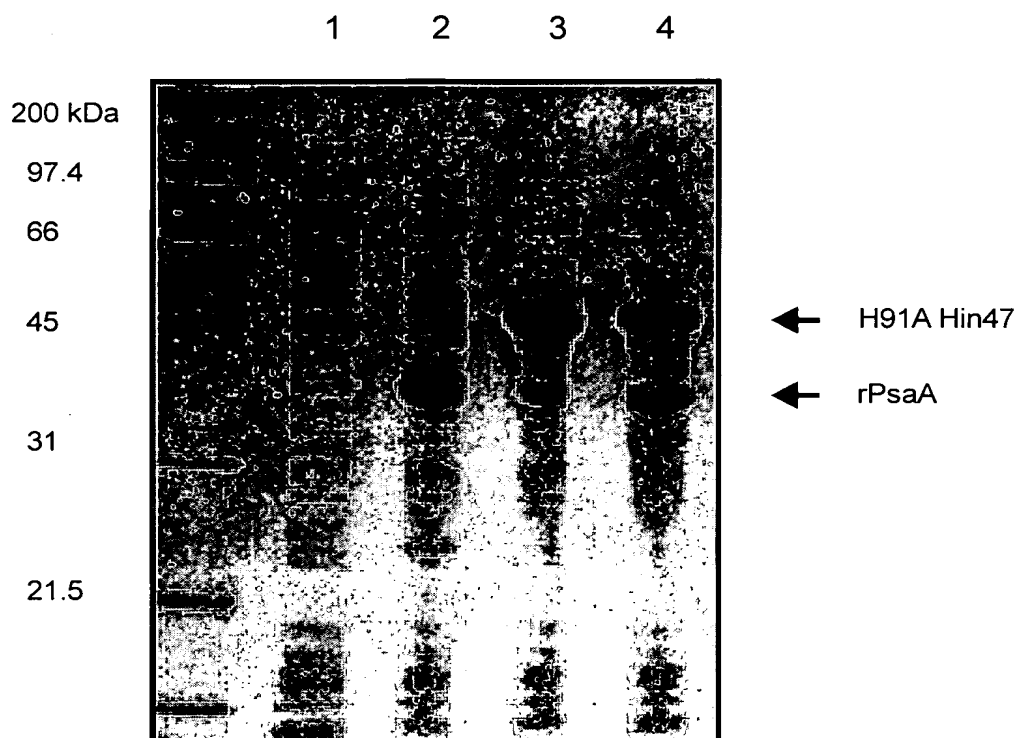
Sense:

5' CGGGATCC CATATG M C A S G K K D T 3' 6851.SL JF 10-20:31
ST 10-20:30

Antisense:

CATGTGAGCTGGCGTTTTTTAAGCTTGGCC
3' GTACACTCGACCGCAAAAAA TTCGAA CCGG 5' 6852.SL JF 10-20:29
ST 10-20:28
Hind III

Figure 14. Production of rPsaA +/- leader and H91A Hin47, when co-expressed



1. H91A Hin47 and rPsaA (+ leader), < > orientation t_0
2. H91A Hin47 and rPsaA (+ leader), < > orientation t_4
3. H91A Hin47 and rPsaA (+ leader), > > orientation t_4
4. H91A Hin47 and rPsaA (- leader), > > orientation t_4

Co-expn fig 14
psaahin

Figure 15. Purification of H91A Hin47 and rPsaA from *E. coli*

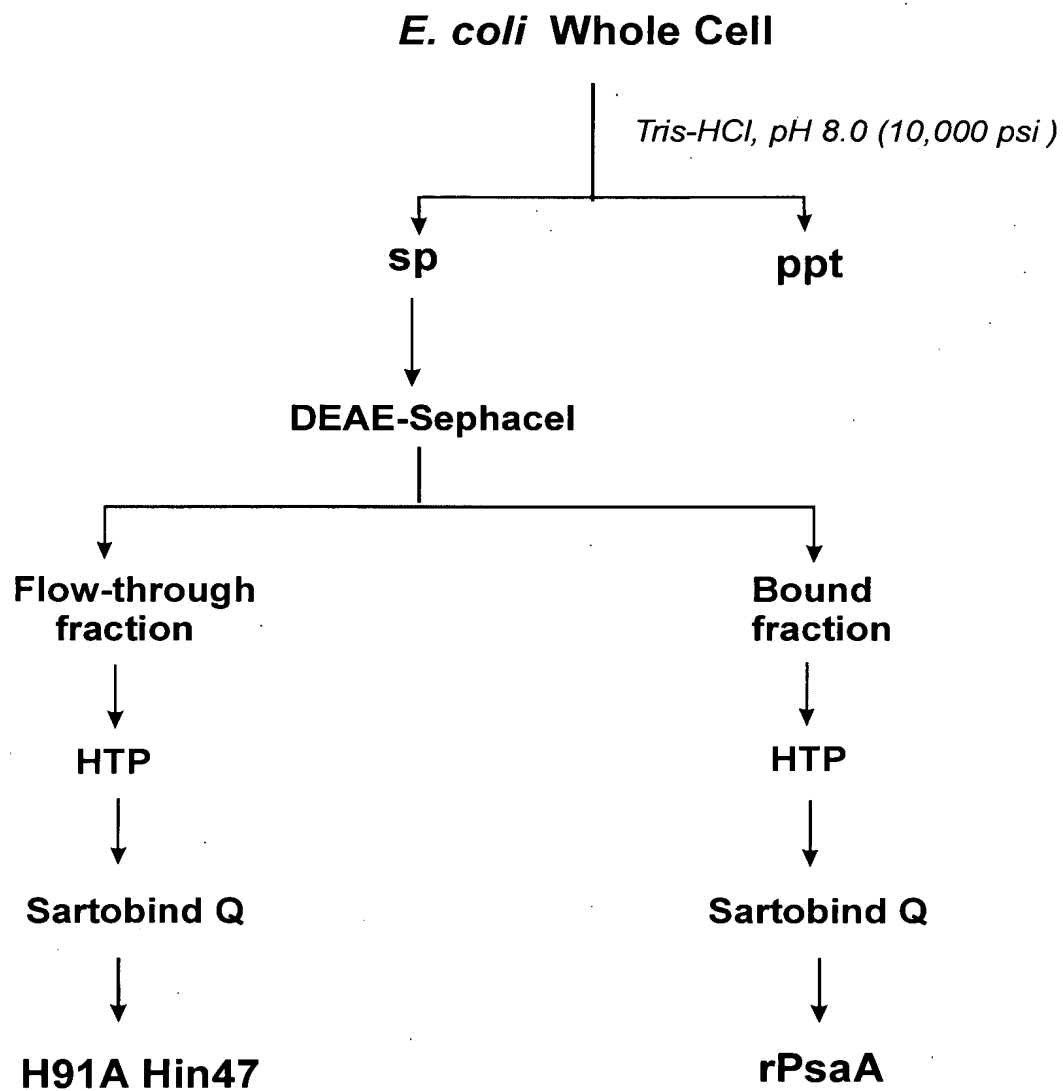
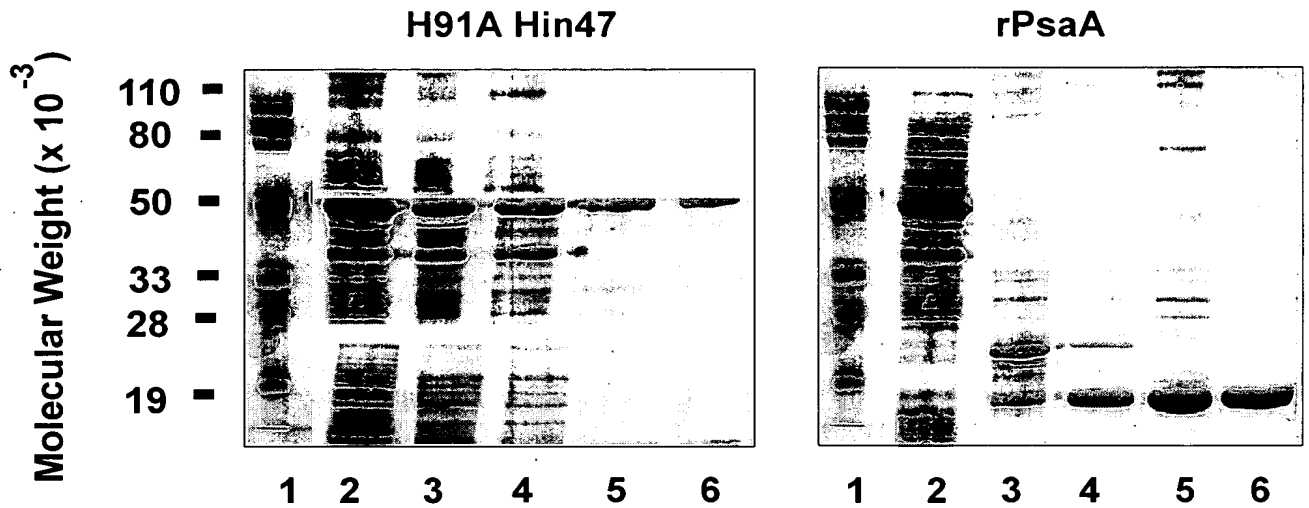
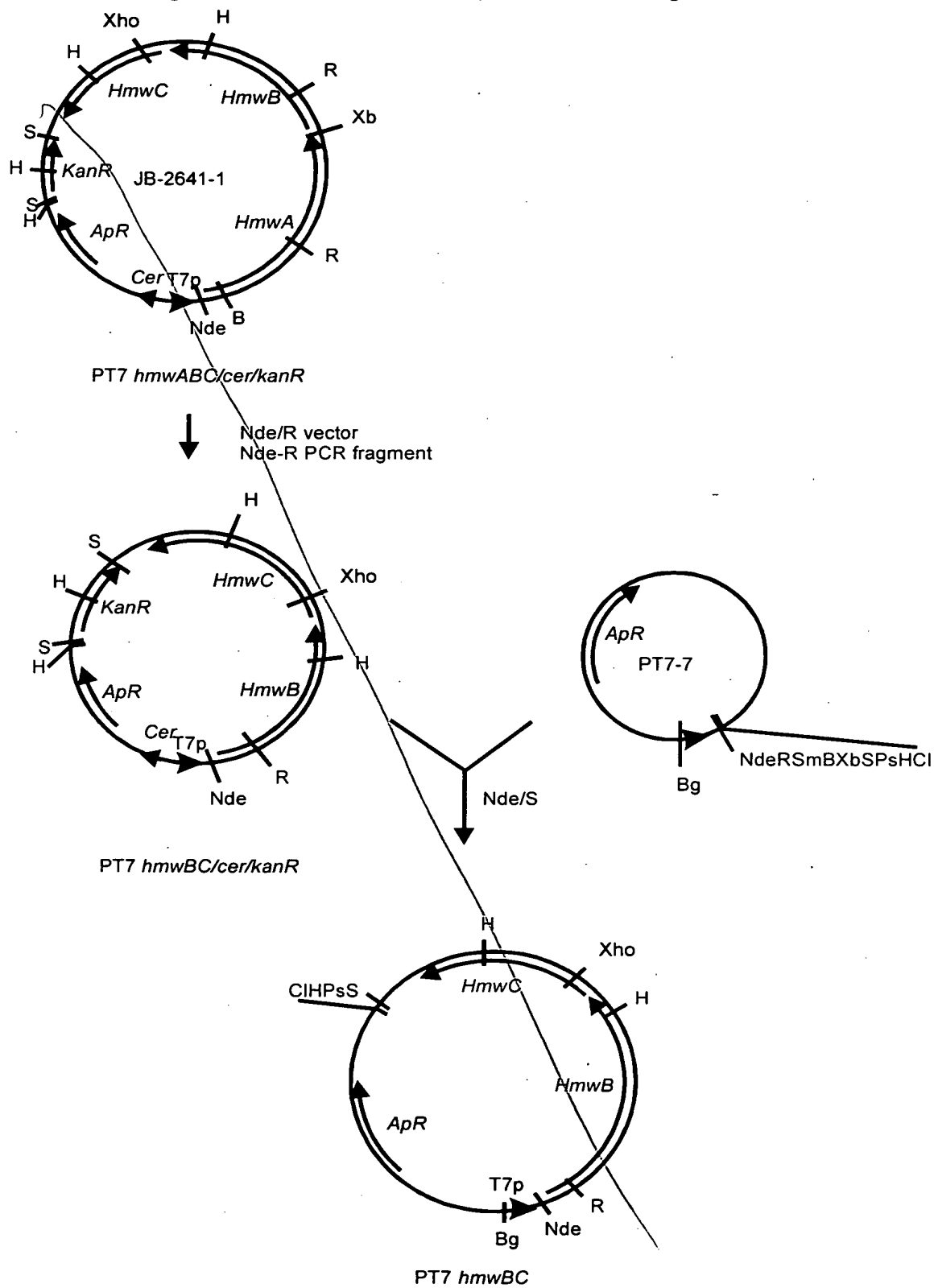


Figure 16. Purification of H91A Hin47 and rPsaA from *E. coli*



1. Pre-stained molecular weight markers
2. *E. coli* whole cell lysate
3. Soluble proteins in 50 mM Tris-HCl, pH 8.0 extraction
4. DEAE-Sephacel column
5. HTP column
6. Sartobind Q membrane

Figure 17A. Construction of a plasmid containing T7 *hmwBC*.



Co-exprn fig 17A
phmwBC

Figure 17B. Oligonucleotide primers for PCR amplification of Nde I-EcoR I 5' *hmvB* fragment.

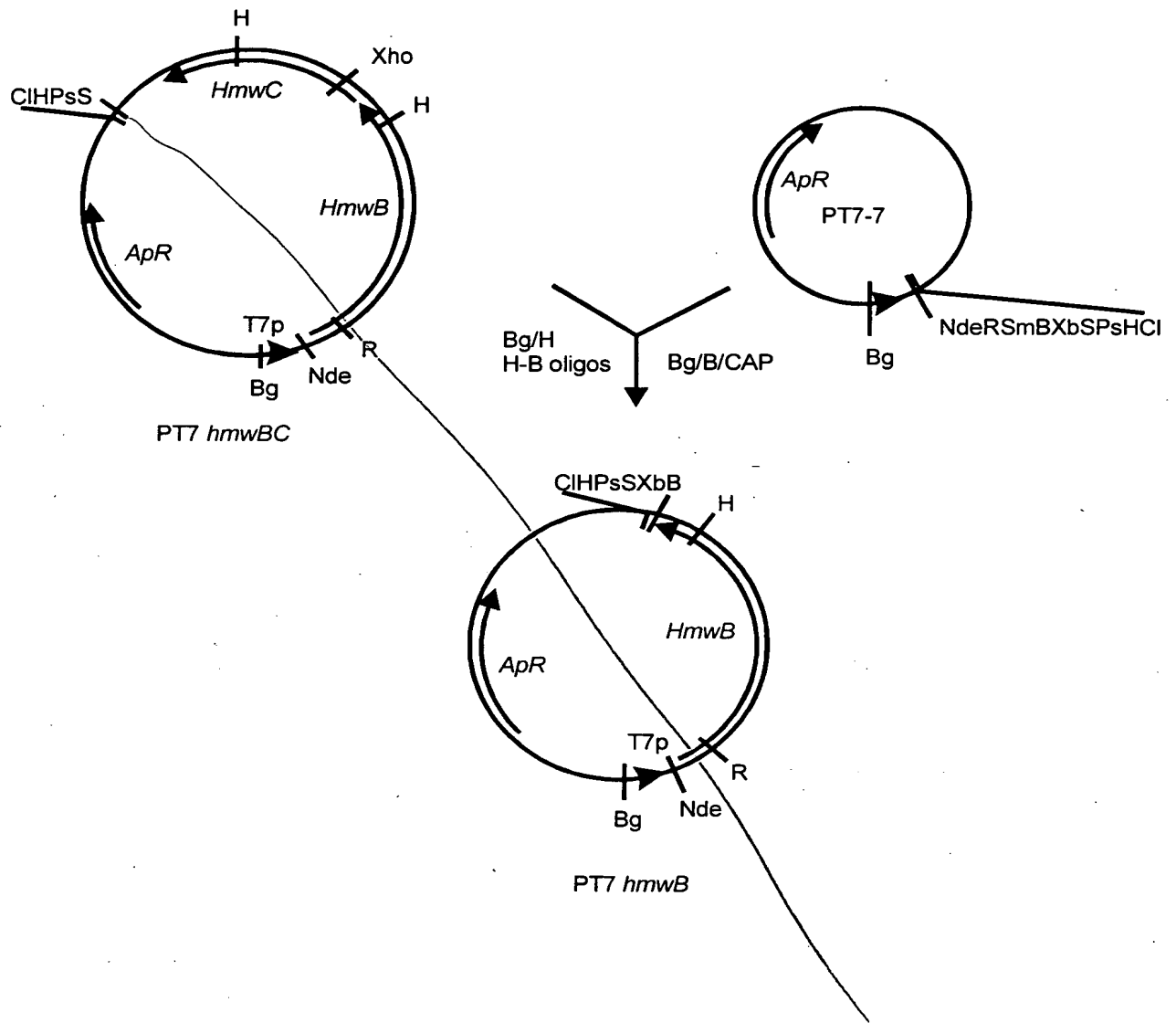
coding strand:

		Nde I			
		M K N I K S R L K L			
5'	GGCCG	<u>CATATG</u>	AAAAATATAAAAAGCAGATTAAACTC	3'	7072.SL
					SEQ ID No: 7
					SEQ ID No: 6

non-coding strand:

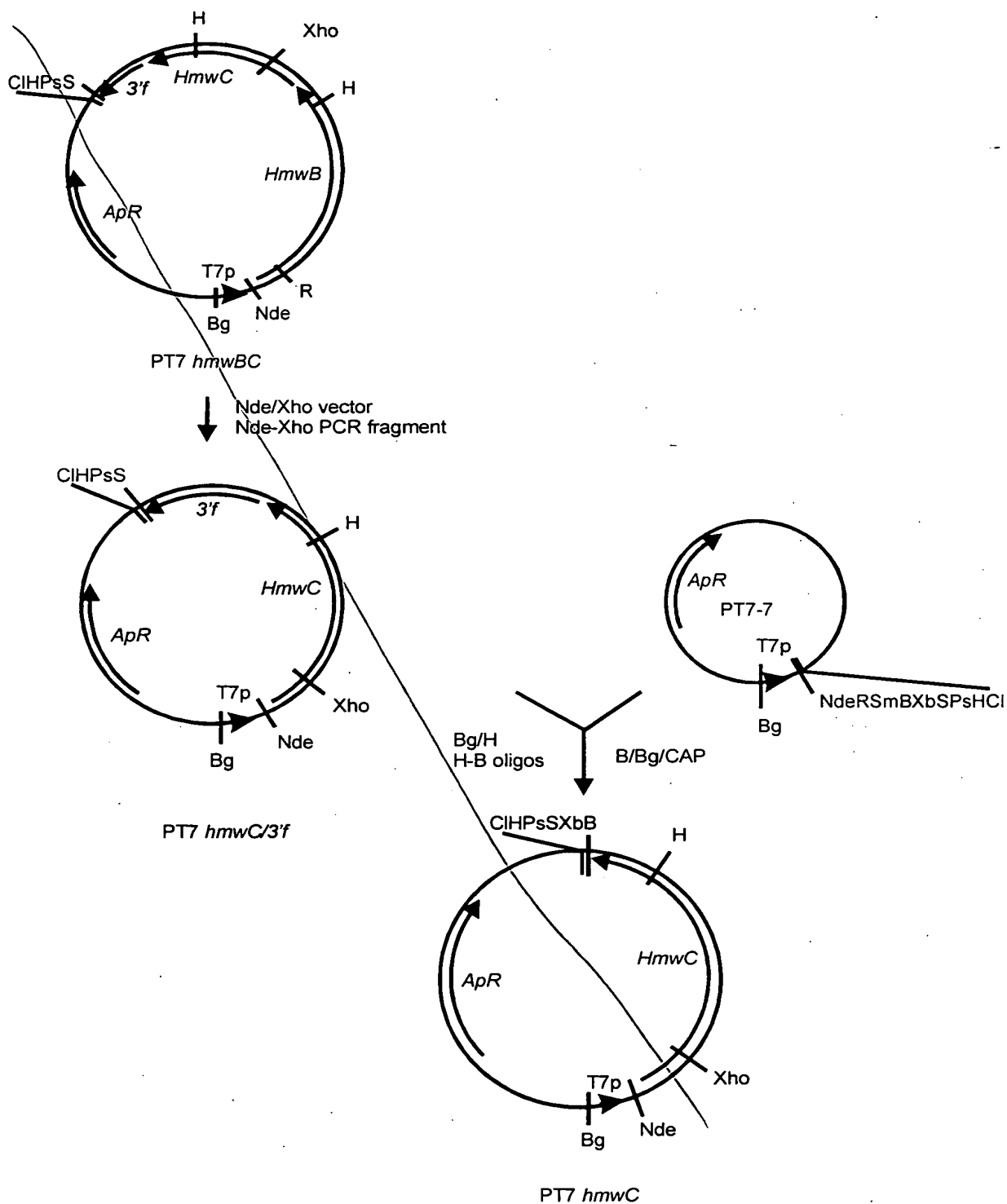
				EcoR I		
		G R Q W F D L R E F N M A				
		GGTCGTCAGTGGTTCGATTGCGT	<u>GAATTC</u>	AAATATGGCA		
3'	CCAGCAGTCACCAAGCTAAACGCACTTAAGTTATACCGT			5'	5950.SL	
						SEQ ID No: 10
						SEQ ID No: 9
						SEQ ID No: 8

Figure 18A. Construction of a plasmid containing *T7 hmwB*.



Co-expn fig 18A
phmwB

Figure 19A. Construction of a plasmid containing *T7 hmwC*.



Co-exprn fig 19A
phmwC

Figure 19B. Oligonucleotide primers for PCR amplification of Nde I-Xho I 5' *hmc* fragment.

coding strand:

		Nde I											
		M	T	K	E	N	L	Q	S	V	P		
5'	GGCCG	CATATG	ACAAAAGAAATTTACAAAGTGTCCA	3'	7077.SL	SEQ ID No:17							
						SEQ ID No:16							

non-coding strand:

				Xho I										
		S	T	S	M	I	A	A	R	E	K	F	Y	SEQ ID No:20
		TCAACTTCAATGATTGCTG	CTCGAG	AAAAATTCTAT										SEQ ID No:19
3'	AGTTGAAGTTACTAACGACGAGCTCTTTTAAAGATA	5'	7078.SL	SEQ ID No:18										

[illegible]